A method to maintain and propagate pluripotent human ES cells

Grant Award Details

A method to maintain and propagate pluripotent human ES cells

Grant Type: SEED Grant

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Investigator:

Name:	Senyon Choe
Institution:	Salk Institute for Biological Studies
Type:	PI

Human Stem Cell Use: Embryonic Stem Cell

Award Value: $760,042

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Progress Reports

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View Report

Grant Application Details

Application Title: A method to maintain and propagate pluripotent human ES cells

Public Abstract:

Human embryonic stem (hES) cells are pluripotent such that they can differentiate into all three germ layers, thus potentially all different types of tissues of the body. Pluripotency is characteristic of only embryonic cells, but it can also be achieved by reprogramming differentiated cells by transferring nuclear contents into unfertilized, enucleated oocytes or by fusing with ES cells. To achieve the initial embryo-like state, it is a pre-requisite to be able to maintain and propagate these ES cells in culture conditions in vitro. Currently, such recipe exists for mouse ES cells. Surprisingly, similar media components for hES cells do not work. This very first technical barrier needs to be overcome in order to realize full clinical potential of stem cell therapy. We propose to develop a novel recipe of chemically defined culture media and culture conditions to grow and maintain pluripotency of hES cells. The media we will evaluate are combinatorial mixtures containing only recombinant proteins, chemically synthesizable reagents, or human source factors. To achieve new sets of recombinant protein reagents known to be involved in controlling differentiation and pluripotency of embryo-like cells, we will develop a novel biochemical strategy of producing a set of target protein reagents effectively in test tubes. To screen conditions using these chemically defined components and various culture conditions, we will develop a new cell line containing a reporter gene (GFP) recombined into human Oct4 gene. Human Oct4 gene is the prominent marker for stemness of the hES cells. There are three specific Aims for this proposed study. They are, 1) production of the media components biochemically, 2) development of two Oct4-reporting hES cell lines, and 3) screening of culture media and conditions for maintaining pluripotency of hES cells. These experiments will be carried out in parallel as collaboration between two laboratories {REDACTED}. Once Aims 1 and 2 are completed, we will evaluate these hES cell lines in various culture conditions systematically (Aim 3). In doing these high-throughput assays for functional characterization, we will also conduct screening of known chemical library of selected drugs and metabolites to glean into their potential ability to augment or inhibit actions of the engineered biologic reagents in controlling the growth and pluripotency of hES cells. From the screening using these two cell lines, we will establish the firm method of propagating and maintaining pluripotency of hES cells for subsequent clinical applications.

Statement of Benefit to California:

Establishing the methods to promote and maintain pluripotency of human embryonic stem cells is a groundwork absolutely necessary to facilitate stem cell research in general and to be adopted for its immediate clinical applications. For instance, the proposed study will generate essential data to facilitate biotechnological approaches to scale up to meet the industrial demand of the much needed protein reagents to culture hES cells. These reagents we established are also critical for basic research of developmental and cell biology, structural biology, and drug discovery. So scientific and industrial benefits to California are enormous.

The proposed study requires combination of extensive biochemistry and developmental biology expertise. Development of new techniques and reagents for the maintenance and proliferation of pluripotent hES cells are fundamentally essential in order to fully exploit therapeutic potentials of hES cell therapy. We believe that the prime benefit from results of the proposed study is to add substantially to the body of knowledge on growing, maintaining, and finally guiding hES cells to the differentiated states as needed to develop effective therapeutic means.
